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Effects of Atmospheric CO_2 Enrichment on the Growth and Mineral Nutrition of *Quercus alba* Seedlings in Nutrient-Poor Soil[1]

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ABSTRACT

One-year-old dormant white oak (*Quercus alba* L.) seedlings were planted in a nutrient-deficient forest soil and grown for 40 weeks in growth chambers at ambient (362 microliters per liter) or elevated (690 microliters per liter) levels of CO_2. Although all of the seedlings became severely N deficient, CO_2 enrichment enhanced growth by 85%, with the greatest enhancement in root systems. The growth enhancement did not increase the total water use per plant, so water-use efficiency was significantly greater in elevated CO_2. Total uptake of N, S, and B was not affected by CO_2, therefore, tissue concentrations of these nutrients were significantly lower in elevated CO_2. An increase in nutrient-use efficiency with respect to N was apparent in that a greater proportion of the limited N pool in the CO_2-enriched plants was in fine roots and leaves. The uptake of other nutrients increased with CO_2 concentration, and P and K uptake increased in proportion to growth. Increased uptake of P by plants in elevated CO_2 may have been a result of greater proliferation of fine roots and associated mycorrhizae and rhizosphere bacteria stimulating P mineralization. The results demonstrate that a growth response to CO_2 enrichment is possible in nutrient-limited systems, and that the mechanisms of response may include either increased nutrient supply or decreased physiological demand.

The 'law of the minimum' evolved in physiological ecology both before and after 1840, when Justus von Liebig is credited with its formulation (4). The doctrine that the environmental resource present in least amount determines the amount of plant growth was initially applied to fertilizers; however, the concept was quickly extended to other resources, including light and water. The importance of the amount of nutrients assimilated by the plant, rather than the amount in the soil, was eventually recognized (4). It is now apparent that the concept of limiting factors is simplistic, and interactions between resources can be expected; that is, more than one resource can limit plant growth simultaneously, or the supply of one resource can increase the supply or decrease the demand for another. Temporal and spatial variation in resource limitations within a plant need also be considered.

Interactions between environmental resources are critical to the analysis of the response of forest vegetation to rising levels of atmospheric CO_2. Increased plant growth is a widely documented response to CO_2 enrichment, but application of the law of the minimum suggests that forest trees, which frequently grow in infertile habitats and are limited by nutrient deficiencies, might not respond to higher levels of CO_2 in a fashion analogous to agricultural systems (12, 20). Because the flux of carbon to terrestrial vegetation, especially forest trees, is an important determinant of the airborne fraction of global carbon (2), the interaction between CO_2 and nutrient deficiencies in forest trees has important implications to the global carbon cycle and global habitability in the face of the certainty of rising atmospheric CO_2 concentrations.

The issue of plant responses to CO_2 enrichment under nutrient-limiting conditions has been addressed in several studies. The proportionate increase in DMI[2] of some crop and weed species with CO_2 enrichment was similar or greater in nutrient-stressed plants than in nonstressed plants (10, 23, 28). Tree seedlings receiving adequate water and nutrients have also been shown to have increased growth with CO_2 enrichment (9, 21, 24); however, there have been few studies of tree responses to CO_2 under low nutrient conditions (8, 15, 18). There are several possible mechanisms whereby nutrient limitations to growth may be alleviated with CO_2 enrichment. Adjustments in the distribution of the nutrient pool within the plant or in metabolic requirements could lower nutrient demand, that is, increase the NUE. For example, if the efficiency of ribulose bisphosphate carboxylase is higher under elevated CO_2, less N would be needed per unit DMI (10). Alternatively, nutrient acquisition could increase if fine root or mycorrhizal growth is stimulated, or if the nutrient supply increases through stimulation of biological activity in the soil and rhizosphere (18, 22). In this study, we present evidence that a growth response to CO_2 enrichment can occur in a nutrient deficient woody plant. Some of the aforementioned mechanisms of carbon and nutrient interactions are also considered.

MATERIALS AND METHODS

Plant Culture. One-year-old, dormant, bare-rooted white oak (*Quercus alba* L.) seedlings were obtained from the Forest Keeling Nursery in Elsberry, MO. The seedlings were planted in 10 × 10 × 35-cm tree pots filled with 2.9 kg (oven-dry weight equivalent) of soil. Thirty-two pots, including four without seedlings, were prepared. The soil, which was dug from an unfertilized oak stand in Oak Ridge, TN, is classified as a Typic Dystrochrept with a shaley silt loam A horizon. Fine sand was added (20% v/v) to improve drainage in the pots. The solution pH of the mixed soil was 4.55, the total N concentration was 1.3 mg/g, and extractable (0.05 N HCl + 0.025 N H_2SO_4) ion concentrations

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[2] Abbreviations: DMI, dry matter increment; NUE, nutrient-use efficiency; WUE, water-use efficiency; LAD, leaf area duration.

were: P, 18 μg/g; K, 162 μg/g; Ca, 1130 μg/g; and Mg, 133 μg/g. Wilde (27) considered 2.0 mg/g of total N and 22 μg/g of available P (50 μg/g of P_2O_5) to be adequate for most forest trees. Although the critical levels of nutrients in soil vary with soil characteristics, plant species, and the method of extraction and analysis, the soil used in this experiment can be considered potentially deficient in N and P.

The seedlings were maintained in a growth chamber for 5 weeks at 27°C (day) and 15°C (night), 200 $\mu mol/m^2 \cdot s$ PAR, a 16-h photoperiod, and ambient CO_2 until the buds of most seedlings were breaking dormancy. Sixteen pots were then randomly assigned to either ambient or elevated CO_2 treatments and placed in matching growth chambers with controlled CO_2 atmospheres (18). Measurement of plant water use began at this time (d zero). By d 28 four seedlings had not broken dormancy, and these were harvested to determine the initial root and stem dry weight and nutrient contents. It was assumed that these seedlings had not grown or changed in nutrient content since d 0. Errors in this assumption would make only minimal errors in subsequent calculations of DMI and nutrient uptake. The remaining seedlings, which were exhibiting rapid shoot elongation and leaf expansion, were maintained in the growth chambers for the duration of the experiment. Temperature in the chambers was 25°C (day) and 7°C (night). The RH was ~65% in the day and near saturation at night. Radiant flux (PAR) at the top of the canopy was 660 $\mu mol/m^2 \cdot s$, with a 14-h photoperiod. CO_2 was injected automatically to maintain the atmospheric concentration in one chamber at 700 μl/L; the actual mean daytime concentration during the experiment was 690.5 ± 27.9 μl/L. The CO_2 concentration in the other chamber varied diurnally and seasonally according to local trends in the ambient CO_2 level (18); average daytime concentration during the course of the experiment was 362.4 ± 15.5 μl/L. The CO_2 concentration in the chambers was monitored with a computer-controlled IR gas analyzer (Anarad AR500RN), calibrated against a CO_2 mixture from the National Bureau of Standards. Chamber assignments and the associated CO_2 levels were switched after 21 weeks.

The watering remine was designed to provide a measure of total plant water use and prevent leaching losses of nutrients from soil. On d zero all pots were watered to the drip point with distilled H_2O, allowed to drain completely, and then weighed. At intervals of 2 to 3 weeks during the remainder of the experiment, the pots were weighed, watered, and reweighed. Nylon mesh on the soil surface minimized the evaporation of water from soil, which was estimated by weighing pots of soil without plants. During the intervals, the pots were irrigated with measured amounts of distilled H_2O to maintain pot weights between ~85 to 95% of the predetermined weight at saturation. The small amount of water that occasionally dripped through the pots was collected and returned to the pots. No nutrients were added to the soil at any time during the experiment.

Harvests. One-half of the plants (six per treatment) were harvested after 20 weeks, and the remainder were harvested after 40 weeks. The shoots were excised, leaves were removed and counted, and their area (one side) was determined with a Li-Cor 1600 Area Meter. Leaves and stems were oven dried (70°C) and weighed. In the second harvest, dormant buds that had formed during the experiment were counted and weighed separately. Abscised leaves were collected, dried, and weighed on a treatment-wide basis. The block of soil was removed from the pot and carefully broken apart. A sample of fine roots with adhering soil was removed for characterization of rhizosphere bacterial populations. Soil samples were collected from throughout the block and frozen. The root systems were washed from the remaining soil and frozen until a mycorrhizal assessment was completed. Roots were then oven-dried, separated into fine roots (<1 mm diameter) and larger roots (primarily the tap root), and weighed.

The frozen soil was sieved (2.36 mm) and triplicate 12-g samples were weighed in 100-ml plastic bottles for extraction of 'leachable' nutrients. The dry weights of the samples were estimated from the ratio of fresh to dry weight (100°C) of equivalent samples. The soil was shaken with 50 ml of 0.01 M NaCl for 30 min, the extract was filtered, and the residue was rinsed with an additional 10 ml of NaCl solution. The extract and NaCl blanks were kept frozen until subsequent nutrient analysis. Leaching was mimicked with the low molarity NaCl extraction instead of distilled H_2O to stabilize ionic strength and minimize the influence of short-term fluctuations in ion exchange (DD Richter, personal communication). The remainder of the sieved soil was air dried and used for nutrient analysis. Soil for nutrient, leachate, and bacterial analyses was also collected from pots without plants.

Rhizosphere Characterization. 'Rhizosphere' was defined as the rhizoplane and adhering soil. The root samples comprised nonsuberized root tips ~5 cm long (~500 mg total) taken from several sites on the root ball. The samples were shaken for 10 min in 50 ml of sterile distilled H_2O with Tween 80 to disperse bacteria. Aliquots of the suspension, which constituted a 10^{-2} dilution of the rhizosphere, were preserved with formaldehyde (final concentration 5% v/v) (6). For the first harvest, 500-μl aliquots of a 10^{-3} dilution were concentrated on a prestained (Iraglan Black), 0.2-μm polycarbonate filter (Nucleopore) and then stained with acridine orange (6). Interference from stained clay particles and organic matter probably led to a uniform overestimation of bacterial populations in all treatments. In the second harvest, bacteria in 100-μl aliquots of the original 10^{-2} dilution were stained with fluorescein isothiocyanate (1), and very little interference was encountered. The filters were mounted on slides and examined under a Nikon Fluophot epifluorescence microscope. The number of bacteria, which fluoresced bright red or green, was counted in 15 microscope fields from each of two replicate slides. The data were expressed as the number of bacteria per unit dry weight of rhizosphere.

Mycorrhizal colonization of roots was assessed under a dissecting microscope. Ectomycorrhizae were counted based on morphological characteristics, including the presence of a well-developed fungal mantle, bifurcation, increased diameter, or absence of root hairs (26). The data were expressed as the number of mycorrhizae per cm of root length, which was calculated by a grid intersect method (17). Mycorrhizal tips were counted in the first harvest and mycorrhizal short roots in the second harvest.

Nutrient Analysis. Dried leaves, stems, fine roots, and tap roots from the initial, 20-week, and 40-week harvests were ground in a Wiley mill and analyzed for concentrations of N, S, P, K, Ca, Mg, Mn, Fe, Al, B, Cu, Zn, and Sr at the Soil Testing and Plant Analysis Laboratory of the University of Georgia. Nitrogen was determined by Autoanalyzer, sulfur by a LECO SC-132 Sulfur Determinator, and other elements by inductively coupled plasma and carbon-arc spark emission spectroscopy using standards traceable to the National Bureau of Standards. The soil leachate samples (NaCl extracts and blanks) were analyzed for NH_4^+, NO_3^-, P, K, Ca, Mg, Mn, Fe, Al, B, Cu, Zn, Na, Pb, Cd, Ni, Cr, and Mo. Soil samples were analyzed for total N and pH and extracted with 0.05 N HCl + 0.025 N H_2SO_4 for analysis of P, K, Ca, and Mg.

Data Analysis. The nutrient content of plant tissue was calculated from nutrient concentrations and corresponding tissue dry weights for each plant component. The total nutrient uptake by each plant was calculated as the sum of tissue contents (including abscised leaves) minus the average amount in seedlings harvested at the beginning of the experiment. DMI was calculated from total plant dry weight (including abscised leaves) minus the average initial dry weight. Average values for abscised

leaves per plant were obtained from the composite sample from each treatment. Whole-plant WUE was calculated as the cumulative water use per plant divided by DMI. Nutrient concentrations of leachate samples were multiplied by total leachate volume (60 ml), corrected for nutrient content of the NaCl blanks, and divided by dry weight of the soil sample. All data were tested statistically by *t*-test, except for leaf abscission data, for which no statistical test was possible.

RESULTS

Shoot extension growth and leaf expansion were completed within the first 6 weeks of the experiment, and there was only one growth flush. Visual symptoms of probable nutrient deficiency (*e.g.* leaf chlorosis) were apparent on most seedlings by 8 weeks. Foliar analysis at 20 weeks indicated a severe N deficiency; the mean concentration of N in leaves in both CO_2 treatments was 17 mg/g, which is less than the deficiency level of 22.2 mg/g given by Mitchell and Chandler (16) for *Q. alba*. During the second 20-week interval of the experiment, the deficiency symptoms developed further and many leaves senesced and abscised. Foliar N concentrations at 40 weeks was 12.0 mg/g in ambient CO_2 and 9.7 mg/g in elevated CO_2. The concentrations of other mineral nutrients in the leaves were in the 'medium' range for *Quercus* spp. (14) and were presumed not to have been deficient.

There was no growth response of any plant component or whole plants to CO_2 enrichment during the first 20 weeks (Fig. 1). The plants grown in ambient CO_2 did not increase in mass during the second 20 weeks, but those in elevated CO_2 continued growing. The final dry weight in elevated CO_2 was 85% greater than in ambient CO_2 (Fig. 1). The greatest proportionate increase because of CO_2 enrichment after 40 weeks was in fine roots (111%), and the greatest absolute increase was in tap roots (93%). There was no evidence of dead or decaying roots in the soil, but the possibility cannot be excluded that the difference in fine root biomass was a reflection of differences in turnover rate. Root to shoot ratio increased from 3.2 to 4.3 with CO_2 enrichment. Because shoot growth in white oak involves the elongation of the preformed stem units and leaf primordia in the resting buds, it is not surprising that the increase in stem dry weight (33%) was less than the increase in root dry weight, and there was no difference in leaf production. However, there was twice as much leaf abscission from plants grown in ambient CO_2; therefore, the dry weight of retained leaves was significantly greater (82%) in elevated CO_2. The number of buds formed during the 40 weeks and the average dry weight per bud both were increased with CO_2 enrichment; the total dry weight of buds was 96 mg in elevated CO_2 and 60 mg in ambient CO_2, significantly different at $P < 0.05$. CO_2 enrichment did not affect specific leaf area.

Water Use. Total water use was not significantly different ($P = 0.24$) in plants grown in elevated *versus* ambient CO_2 (Fig. 2). Whole-plant WUE (DMI per unit water used) was 3.83 ± 0.67 g/L in ambient CO_2 and 6.10 ± 0.80 g/L in elevated CO_2, significantly different at $P < 0.001$.

Nutrient Relations. There were no significant differences in elemental composition in plants harvested after 20 weeks, but there were substantial differences in some elements at 40 weeks. There were three patterns of nutrient uptake (Table I): (a) the total uptake of N, S, and B per plant (including that lost in leaf abscission) over 40 weeks was similar in ambient and elevated CO_2 (difference < 20%); therefore, uptake per unit DMI and whole-plant concentration were significantly less in plants in elevated CO_2; (b) the uptake per plant of Ca, Mg, Mn, Zn, and Sr was at least 35% greater in elevated CO_2, but the increase was less than the proportionate increase in DMI; and (c) the uptake per plant of P, K, Fe, Cu, and Al was enhanced by CO_2 enrichment at least as much as was DMI; consequently, the concentrations of these elements were not significantly different in plants

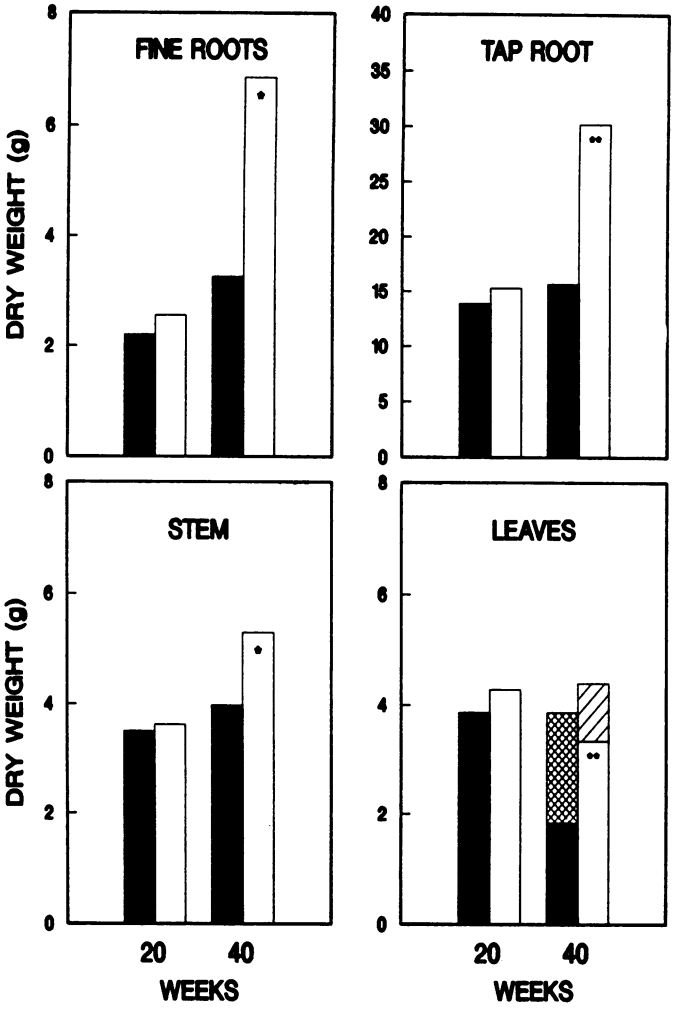

FIG. 1. Dry weight of fine roots (<1 mm diameter), tap roots, stems, and leaves of white oak seedlings after 20 and 40 weeks in 362 (■) or 690 (□) μl/L CO_2. Total leaf production at 40 weeks is divided into abscised (hatched bars) and retained leaves. *, $P < 0.05$; **, $P < 0.01$.

at different levels of CO_2.

The concentration of N in plants grown in elevated CO_2 was significantly less in all tissues (Table II), but relative to the whole-plant pool of N, plants grown in elevated CO_2 had proportionately more N in fine roots and attached leaves, less in tap roots, and less was lost in leaf abscission. Although whole-plant concentrations of B and S were also significantly lower in elevated CO_2, their patterns of distribution differed from that of N. There were greater proportions of B in both fine roots ($P < 0.05$) and tap roots ($P < 0.01$), and no difference in the proportions in stems or leaves. There were no significant differences in the proportionate distribution of S or any other nutrient.

The CO_2 concentration in which the plants were grown did not affect the leaching of elements from soil (NaCl extraction), except that there was 25.0% less K in leachate in elevated CO_2 at 40 weeks ($P < 0.04$). The concentrations of extractable nutrients in soil at 20 and 40 weeks were similar to those in initial soil samples, except that soils in which plants were grown (regardless of CO_2 concentration) had lower amounts of extractable P and K. The soil solution pH increased from the initial 4.55 to 4.82 at 20 weeks and to 4.95 at 40 weeks in pots with plants, and there was no effect of CO_2. Of the elements analyzed in both soil and plants, P and K were the only ones for which plant uptake was a significant fraction of the total available nutrient pool (Table III), assuming that nutrient concentrations in soil

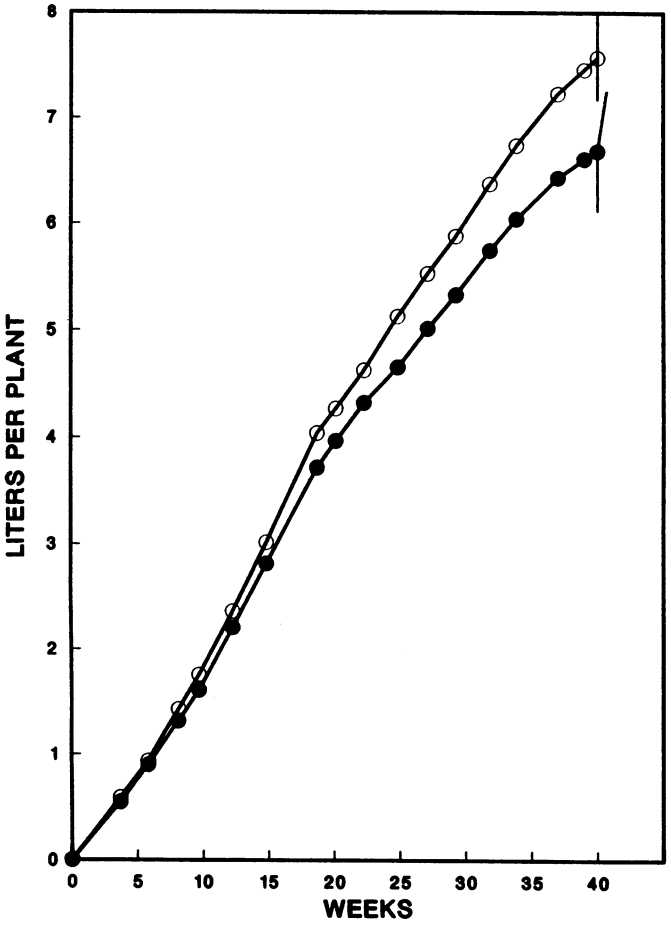

FIG. 2. Cumulative water use per plant during 40 weeks in 362 (●) or 690 (○) μl/L CO_2. Error bars represent ± SE.

extracts approximated the amounts in soil available for plant uptake. The increase in plant uptake of P in elevated CO_2 was not associated with a concomitant decrease in extractable or leachable P in the soil. Therefore, the total amount of available P in the soil-plant system was significantly greater in elevated CO_2. The increase in plant uptake in elevated CO_2 was apparently associated with an increase in the mineralization of organic P or the solubilization of inorganic P. The turnover rate of K in soil is much slower than that of P (3) and was probably not a factor in this experiment. Hence, the increase in plant uptake of K in elevated CO_2 was associated with a decline in the soil pool and a significant decrease in leachable K in the soil system.

Rhizosphere Relations. CO_2 enrichment did not significantly affect the population density of rhizosphere bacteria or ectomycorrhizal colonization of the white oak seedlings (Table IV). The total numbers of ectomycorrhizae and rhizosphere bacteria per plant or pot cannot be calculated, but it can be assumed that total mycorrhizae and bacteria populations in elevated CO_2 increased in concert with the large increase in fine root dry weight.

DISCUSSION

The white oak seedlings in this experiment were grown in a forest soil of low nutrient status with no added fertilizer, and thus the plants became severely N deficient. The concentration of N in leaves was well below the level of 22.2 mg/g given as a minimum for adequate growth of *Q. alba* (16) and below the 'medium' range for *Quercus* spp. (14). Similar levels of N deficiency can occur in *Q. alba* trees in unmanaged forests (11, 30). A comparison of experimental data with literature values for a 'critical nutrient concentration' cannot by itself establish that an element was limiting plant growth (14); however, coupled with the low soil N content and the visible symptoms of nutrient stress, it is probable that N was deficient in the plants of this experiment.

Despite the N deficiency, a significant growth enhancement occurred in response to CO_2 enrichment. The enhancement was manifest primarily in root growth. CO_2 enrichment has been reported previously to affect root growth more than shoot growth in some woody plants (8, 9, 15, 18), but the root to shoot ratio of *Pinus taeda* and *Liquidambar styraciflua* seedlings was not affected (24). Because the ratio of root to shoot growth can increase with nutrient stress (5), roots of nutrient-deficient plants may be more likely to respond to CO_2 enrichment. Caution is needed, however, in interpreting the pattern of growth partitioning in the present experiment. The 'fixed growth' habit of *Q. alba* precluded a shoot elongation response to CO_2 concentration,

Table I. *Effect of CO_2 Concentration on Nutrient Uptake*

Nutrient uptake per plant was calculated as the amount in plants (including abscised leaves) after 40 weeks minus the average amount in dormant plants at the start of the experiment. Significance values (P) indicate the probability of no effect of CO_2 concentration. Categories of nutrient use were defined by grouping percentage increases and significance (P) levels.

Category	Element	Nutrient Uptake				Uptake: Growth Ratio			
		362 μl/L	690 μl/L	Increase	P	362 μl/L	690 μl/L	Increase	P
		mg/plant		%		*mg/g DMI*		%	
I	N	155.7	172.3	10.7	>0.2	9.12	4.48	−50.9	0.002
	S	18.0	21.4	18.9	>0.2	0.55	1.09	−49.6	0.019
	B	0.437	0.491	12.3	>0.2	0.027	0.013	−50.9	0.004
II	Ca	157.6	259.5	64.7	0.016	8.97	6.76	−24.6	0.005
	Mg	23.0	33.2	44.0	0.069	1.34	0.85	−36.6	0.001
	Mn	15.8	24.3	53.0	0.016	0.982	0.647	−34.1	0.084
	Zn	0.826	1.130	36.8	0.147	0.048	0.030	−38.0	0.013
	Sr	0.404	0.624	54.6	0.143	0.026	0.016	−30.6	0.006
III	P	25.1	54.7	117.9	0.003	1.39	1.41	1.6	>0.2
	K	79.9	179.2	124.4	0.003	4.66	4.59	−1.6	>0.2
	Fe	1.58	4.01	154.4	0.009	0.077	0.106	37.9	>0.2
	Cu	0.209	0.485	131.8	0.060	0.012	0.013	5.9	>0.2
	Al	7.77	15.37	97.9	0.017	0.408	0.414	1.6	>0.2

Table II. *Effect of CO_2 Concentration on Concentration and Proportionate Distribution of N in White Oak Tissue*

Component	Tissue Concentration			Proportion of Total Plant Content		
	362 µl/L	690 µl/L	P	362 µl/L	690 µl/L	P
	mg N/g dry wt			%		
Fine roots	9.3	7.7	0.05	12.3	21.0	0.06
Tap root	9.0	4.4	0.001	58.5	49.7	0.09
Stem	8.3	6.1	0.01	13.8	12.4	>0.20
Attached leaves	12.0	9.7	0.02	7.2	13.0	0.03
Abscised leaves	9.4	9.2		8.2	3.9	

but the increase in bud mass in plants grown in elevated CO_2 suggests that these plants might have exhibited an increase in shoot elongation in the next growth cycle. The dry weight of new shoots would be attributable in part to carbon compounds that were translocated from storage pools in the tap roots. The large increase observed in this experiment in the root to shoot ratio of plants grown in elevated CO_2 may be a temporary response.

The growth increase in elevated CO_2 was associated with a large increase in WUE (DMI per L water used), although total water use per plant was not affected. An increase in WUE is a common response to CO_2 enrichment, which is attributed to a reduction in transpiration through stomatal closure while CO_2 assimilation rate is maintained or increased because of the larger diffusion gradient (20). The effect of CO_2 enrichment on WUE has been determined primarily in short-term measurements of gas exchange at the leaf surface. These data have occasionally been extrapolated to conclude that vegetation will use less water as the atmospheric CO_2 concentration increases. The present experiment demonstrates that increased WUE is not necessarily associated with decreased water use.

The basis for the growth enhancement of seedlings in elevated CO_2 was in part the greater LAD of these plants, which was a result of reduced leaf abscission and not increased total leaf area production. This conclusion is consistent with the observation that growth enhancement did not occur until the second 20-week period when leaf abscission began. Leaf senescence and abscission were probably a consequence of N deficiency. The effect of CO_2 on leaf retention, however, cannot be attributed to differences in N (foliar N concentrations were lower in plants grown in elevated CO_2) but may have been related to a higher rate of photosynthesis and production of senescence-retarding growth regulators (13) in leaves of CO_2-enriched plants. Although rigorous quantitative growth analysis is not possible with these data, an increased net assimilation rate in elevated CO_2 during the second harvest interval probably also contributed to growth enhancement. The maximum LAD in elevated CO_2, assuming that no leaves abscised until the last day, was 1.20 $m^2 \cdot$ week; the minimum LAD in ambient CO_2, assuming that all of the leaf abscission occurred on the first day of the interval, was 0.47 $m^2 \cdot$ week. This maximum difference in LAD is not sufficient to explain the large difference in DMI, thus implicating an enhancement of net assimilation rate in response to CO_2 enrichment.

The interaction between CO_2 enrichment and nutrient utilization varied with different nutrients. The three patterns of nutrient uptake, which actually represent a continuum in response, probably resulted from differences between the various elements as to physiological demand by the plant, availability in the soil, and plant mechanisms for altering the availability in the soil. The interaction between CO_2 and N is the most relevant to the interpretation of this experiment and to the general issue of the responses of forest trees to elevated CO_2, because N was the most deficient nutrient in the white oak seedlings and commonly is a limiting nutrient in forests (7, 13). Although seedlings grown in elevated CO_2 were significantly larger than those grown in ambient CO_2, the total N content of the seedlings was similar. The plants apparently took up all of the available N in the pot, and plant physiological processes such as root exudation, which could increase nutrient availability (5), or fine root growth, which could increase soil exploration and nutrient acquisition (5), were not effective in overcoming N deficiency. These results with N do not support the hypothesis that uptake of limiting nutrients from a nutrient-depleted soil will increase as atmospheric CO_2 concentrations rise (18). Instead, the oak seedlings in elevated CO_2 had a higher NUE with respect to N; that is, the concentration of N in the plants, or more appropriately, the ratio of N uptake to DMI, decreased. One of the manifestations of the increase in efficiency was a more favorable distribution within the plant of the limited pool of N. Seedlings grown in elevated CO_2 had greater proportions of the N pool in metabolically active tissue (fine roots and attached leaves) and less in storage tissue and abscised leaves.

The higher proportion of N in fine roots can be accounted for by considering C-N interactions (7). In ectomycorrhizal plants inorganic N is captured from the soil by the fungus, which releases amino acids to the roots. The amino acids are used preferentially in root processes as long as carbon supply from the shoot is sufficient. When carbon becomes limiting, N is transported out of the roots toward the shoots. In oak seedlings in high CO_2 there was a greater supply of carbon to root systems than in plants in ambient CO_2, a result of higher LAD and whole-canopy photosynthesis. Root processes in the enriched plants were, therefore, less carbon limited, and N was utilized in fine

Table III. *Distribution of Available Pools of P and K Between Soil, Soil Leachate, and Plant Uptake after 40 Weeks of Plant Growth*

Soil blanks were pots of soil without plants. Significance of effect of CO_2 concentration: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Element	Treatment CO_2 Level	Amount of Element				Percentage of Total	
		Soil (extractable)	Soil leachate	Plant uptake	Total	In plant	Leached
	µl/L	*mg*					
P	362	39.0	1.0	18.8	58.9	29.6	1.8
	690	34.2*	1.2	54.7***	90.1**	59.9***	1.4
	Soil blank	49.1	1.2		50.3		2.4
K	362	347.2	92.6	79.9	519.6	15.4	17.8
	690	288.4	73.2	179.2	540.9	33.2**	13.6*
	Soil blank	375.6	137.0		512.6		26.8

Table IV. *Effect of CO_2 Concentration on Mycorrhizal and Rhizosphere Bacteria Population Densities*

Mycorrhizal density is expressed as the number of ectomycorrhizae per cm fine root ± SE. Mycorrhizal tips were counted at 20 weeks and mycorrhizal clusters at 40 weeks; therefore, the data are not directly comparable. Rhizosphere bacteria population density is the number of bacteria per ng rhizosphere dry wt ± SE. No differences between CO_2 treatments were statistically significant ($P > 0.20$).

CO_2 level	Mycorrhizal Density		Rhizosphere Bacteria	
	20 Weeks	40 Weeks	20 Weeks	40 Weeks
μl/L	*no/cm*		*no/ng*	
362	19.3 ± 2.9	7.8 ± 2.4	2.85 ± 0.90	1.28 ± 0.39
690	28.4 ± 6.7	8.2 ± 1.4	2.46 ± 0.66	1.10 ± 0.21

root growth rather than transported out toward the tap root and the shoot. The CO_2-enriched plants thus maintained a greater proportion of the total N pool in fine roots compared to plants in ambient CO_2, although the concentration of N was lower due to the dilution effect of increased fine root biomass. The greater proportion of the total N pool in attached leaves in elevated CO_2 is attributable to the reduction in loss of N in leaf abscission. These changes in NUE with respect to N are not offered as mechanisms for the growth responses to CO_2 enrichment, but as a reflection of the changes in carbon assimilation and allocation.

With the exception of N, S, and B, nutrient uptake rates were higher in plants grown in elevated CO_2, and the increases in uptake of P, K, Fe, Cu, and Al were at least as great as the increase in DMI, such that tissue concentrations were similar in ambient and elevated CO_2. Although the uptake of divalent cations was proportionately less than the increase in DMI in elevated CO_2, and tissue concentrations were somewhat reduced, the amounts of these elements were still in excess of physiological requirements. Increased uptake of K in elevated CO_2 was associated with a decrease in the leaching of K from soil. Reductions in the loss of K from ecosystems have also been associated with plant uptake and accumulation in biomass (25). Various effects of CO_2 concentration on nutrient leaching were also shown in systems with *Liriodendron tulipifera* (18) and *Pinus virginiana* seedlings (15).

The specific stimulation of fine root growth probably was a critical response to CO_2 enrichment in regard to the maintenance of sufficient nutrient concentrations (excepting N) in concert with growth increase. The increase in P uptake was important to the overall plant response to CO_2 enrichment, considering that P availability was low in the soil used in this experiment (and is frequently limiting in forest soils). If the total uptake of P had not increased in elevated CO_2, foliar concentrations of P in those plants would have been reduced to <1.2 mg/g (assuming no change in proportionate distribution), which may represent a physiological deficiency (14). The increased exploration of the soil by fine roots and mycorrhizae is especially important for the uptake of P, which is relatively immobile in the soil (5). Although mycorrhizal colonization was not specifically stimulated by CO_2 enrichment, the total number of ectomycorrhizae was presumed to be greater for plants in elevated CO_2. The available pool of P in many soils is primarily derived from the microbial decomposition of organic matter (29), and this process can be stimulated by rhizosphere bacteria. The rate of mineralization of P was apparently higher in soil-plant systems maintained in elevated CO_2. This may have been a consequence of greater fine root area and associated rhizosphere activity, even though the population density of rhizosphere bacteria was not increased.

Contrary to the results with N, the results with P and other nutrients are consistent with the hypothesis that nutrient availability and uptake in nutrient-poor systems will increase in response to CO_2 enrichment (18). In contrast to these results, there was no response to elevated CO_2 in *Populus euramericana* cuttings and several herbaceous plants when they were grown in sand with low-P nutrient solution (8). The use of sand culture, however, precluded changes in P mineralization, and the conclusion that the interaction of CO_2 with P was 'fully governed by the law of limiting factors' therefore is not appropriate.

The results of this study have similarities with those of a related study with *Pinus virginiana* exposed to elevated CO_2 in open-top field chambers (15). Both studies demonstrated a growth response of tree seedlings to CO_2 enrichment despite the low nutrient status of the soil. The specific changes in nutrient uptake differed, however, in the two contrasting soil-plant systems. In the *P. virginiana* study, N uptake was proportionate to growth over the range of CO_2 concentrations, the uptake of P and K was less than proportionate, and Zn uptake was greater than proportionate.

It is difficult to extend the responses of seedlings in controlled environments over 40 weeks to predictions of the responses of forest trees over their life spans. Nevertheless, this study establishes that growth enhancement from CO_2 enrichment is possible under nutrient-deficient conditions. The mechanisms of response are likely to vary with species, soils, and nutrients. Increases in NUE, as those that occurred in this study with N, could have deleterious effects over the longer term that could limit the growth enhancement from CO_2 enrichment. For example, increased shoot production in the next growing season, which was suggested by increased bud mass, may be limited by the decreased supply of N in storage tissue. The availability of N in soil may decline because of poorer litter quality. The relationship of the short-term physiological responses demonstrated in this study to longer-term aspects of forest productivity has been considered elsewhere (19). Carbon-nutrient linkages at both physiological and ecological levels should be an important component in the assessment of the responses of forest vegetation to rising atmospheric CO_2.

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